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A PRELIMINARY STUDY OF *CLAVICEPS PURPUREA* IN CULTURE

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INTRODUCTION

The work with *Claviceps purpurea* Tul. herein considered had in its inception a purely physiological objective resulting from the pressure of economic conditions. It is doubtful if the majority of botanists or laymen have a proper conception of the positive economic value of this fungus. To emphasize this point it will suffice to say that the annual import of ergot into the United States for the six years ending with 1919 has ranged from 58 to 112 tons, with a valuation ranging as high as $208,000. Practically all of this, so far as we are aware, is used in the manufacture of pharmaceutical preparations of great importance in obstetrics.

The great commercial sources of the sclerotia in the past have been Spain and Russia. During the recent war American drug manufacturers experienced a great shortage of ergot, and as a result quotations rose by leaps and bounds. In the early part of 1914, high-grade Russian ergot could be bought for 43 cents a pound, duty paid. During the first part of 1920, quotations on this drug ranged between $5.00 and $6.00 a pound.

Our objective, therefore, in undertaking this study was the growth of the fungus on artificial media to determine if under such conditions the physiological principles extracted from the natural sclerotium could be obtained.

LITERATURE

The literature dealing with the physiology of Claviceps is scant. A fairly complete monograph of the genus by Atanasoff (1) gives but three citations of studies of the fungus in culture. According to this writer, Brefeld (7) is credited with being the first to study *C. purpurea* under artificial conditions. The latter grew his cultures from ascospores germinated on bread soaked in nutrient solution. A brief description of the conidial stage is given. Neither sclerotial formation nor anything apparently homologous thereto was obtained in cultures.

About a dozen years after Brefeld's study, Meyer (12) repeated the experiment, starting his cultures, however, from the "honey-dew stage" on infected rye. This paper gives the most detailed description of any

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so far found, and, because of its bearing on the present work, will be more fully considered later.

Engelke (9), working both with nutrient solution and nutrient agar, furnishes very little detailed information regarding the morphology of the fungus. Brefeld's observations were in general confirmed. Both solid media and nutrient solutions were employed, the former consisting of glucose, ammonium nitrate, mono-basic potassium phosphate, and magnesium sulphate. To this 2 percent agar was added for solid media. The formation of "microsclerotia" on solid media with lowered temperature is noted, but no description or illustration gives any clew as to their nature. This paper is of interest because of the proposal for growing the fungus in culture for the purpose of obtaining a growth possessing the physiological activities of the natural sclerotium. So far we have found no later contribution on this point.

Engelke observed no honey dew in artificial cultures, and regarded the excretion as an increase of stigmatic fluid due to parasitic stimulation and not an excretory product of the sphacelial stage.

Brown and Ranck (8), in a study of forage poisoning, inoculated bean pods, peptone agar, and Paspalum heads with portions of *Claviceps Paspali* sclerotia, securing pure cultures upon the bean medium. The fungus penetrated the pods, but no reproductive bodies were noted. The germination of this species of Claviceps and the production of the sphacelial stage as described by these authors are closely analogous to the conditions in *C. purpurea* as noted by the workers previously cited.

Meyer was guided in the preparation of his nutrient solutions by the chemical composition of the immature rye seed. His liquid medium contained starch, glucose, albumin-peptone, asparagin, di-potassium phosphate, magnesium sulphate, potassium chloride, and ammonium sulphate in proportions to make the ash constituents of the combination approximate those of the rye seed as determined by the organic analysis of Nowacki. The fungus was studied in such solution cultures, or the liquid was added to bread or to cotton. Meyer's descriptions and illustrations of the fungus in culture conform in all essentials with those of Brefeld, but more details are given in the later work. Emphasis is laid upon the rapid growth and prolific spore production, with early germination after abscission. The development of cross walls in the spore before maturity, the presence of two bipolar oil drops before abscission, and the grouping of spores about terminal hyphae or branches after abscission are all noted.

Meyer observed two distinct hyphal stages; in one, the cells were well filled with strongly granulated, fat-containing protoplasm; in the other, the cells were for the most part poor in plasma content, with numerous hyaline vacuolate areas. The latter condition he correlates with advanced hyphal age.

Cultures resulting from inoculation with spores of the honey-dew stage

showed spore development in three weeks. A thick mycelial pellicle was obtained on nutrient solutions in 2½ months. Aerial mycelium was produced on bread, potato, and other organic media with inocula from previous cultures. Hyphae on solid media developed spores in scantier amount than those in water cultures. The great viability of the conidiospores was commented upon, and Meyer raises the question whether such spores may not winter over. No heliotropism was noted at any stage of growth.

The plications on solid media noted by Brefeld were confirmed, together with the successive change in pellicle color from yellow to brown, but Meyer does not record the deep violet or purple stage which Brefeld emphasizes. A resting period of the fungus was indicated by a complete cessation of vegetative activity in mid-December of cultures started in July. Renewed activity with continued increase of mycelial growth was observed after a two months' rest period. Cultures in the resting condition gave off, when opened, an odor of trimethylamine.

An attempt was made to distinguish a morphological differentiation between growths on solid and on liquid media. In the former the mycelium was characterized by compact parallel grouping of the hyphae in bundles, whereas in liquid cultures the mycelium branched profusely and grew in all directions.

With respect to sclerotial formation or a tendency thereto, Meyer sustains Brefeld in finding no indications in this direction, even in cultures held for a year. The hyphal elements of the resting pellicle were not pseudo-parenchymatic, as are the cells in a cross section of a sclerotium, but were grouped as masses of short cells, rich in fat. On the older convoluted pellicles a closely compacted parallel hyphal development was noted. From these strands, basidiospore-like elements with terminal spores developed at right angles. The resting stage is regarded as purely conidial or basidial.

Experimental

It was impossible to begin the study here reported with ascospores, as work was started in June, when viable sclerotia of the preceding year's formation were not available. The attempt was therefore made to secure a growth from sclerotia just forming on that season's rye crop. A few rye heads with fairly well developed but still immature sclerotia were obtained in Northern Indiana in June, 1919. These were readily cut transversely with a sterilized razor, the tissue being firm but not hard. Discarding the exposed tissue, portions were removed under sterile conditions from the interior of the sclerotium and transferred to agar.

The medium used in the preliminary work was 2 percent agar (showing a reaction of + 15 Fuller's scale) with decoctions made from rye seed, rye screenings, and germinated rye. The tissue thus obtained was transferred to Petri dishes. Transplants which showed no contamination at

the end of a week were transferred to tubes on rye-screenings agar, standing at room temperature.

Sixteen days after transfer to test tubes a very slight growth was indicated, and three weeks later this was distinct in character and identical in all tubes. It had neither bacterial nor gross mycelial characters, resembling in general appearance some Actinomyces in culture. Under low magnification (20 diameters) it showed a smooth, glistening, greyish-white, gelatinous-like surface, peculiarly vermiculated. Repeated transfer to fresh tubes resulted in all cases in growth of similar nature, sometimes progressing directly from the inoculum, and at times first appearing on the surface of the slant at some distance from the point of inoculation as small pustules scattered over the surface of the slant, finally coalescing to form an area similar to the parent growth.

After several transfers the work was interrupted, and, pending resumption of the study, the tubes were sealed in September, 1919, and kept at room temperature. It may here be noted, bearing in mind Meyer's observations of the longevity of conidiospores, that these cultures retained their viability for over a year. This is also in line with Stäger's (14) findings that the conidiospores are viable after wintering over a period of ten months.

No gross mycelial characters had developed in any tubes up to this time, nor did any appear in a period of more than eight months. The consistent nature of successive transplants warranted the assumption of a pure culture, but no detailed microscopic study had been possible. Whether a pure culture of Claviceps, originating from sclerotial tissue, had been obtained, or merely a fortuitous contaminating organism of a parasite on the sclerotium, remained to be established.[1] In January, 1920, smears from several tubes showed a dense stroma, at the periphery of which short hyphal elements and an occasional conidiospore were evident, but no further work towards identification of the fungus was then possible.

In April, 1920, work was resumed by inoculating Petri dishes and tubes of the three kinds of agar with the preceding September cultures. Typical growths appeared on the third day following inoculation, both in tubes and in dishes.

The primary growth preceding mycelium formation was the same as that noted the preceding summer. Smears showed this to be an almost solid field of oval spores in resting condition and in all stages of germination (Pl. XVII, fig. 2). Occasionally a hyphal thread could be noted, and in the majority of these the protoplasmic contents were distinctly segmented, with rounded ends and vacuolate areas between.

Five days from date of inoculation mycelial development began and

[1] Field inoculations on rye with growths on agar were made in 1920, with apparently positive results. The evidence, however, was rendered circumstantial by loss of identification tags in a severe windstorm.

progressed slowly until checked by the drying down of the media (Pl. XVII, fig. 4). No conidia formation and no sterigmata were noted in the dishes. On the agar slants spores were very numerous.

The cycle from germination to spore formation in the first stage of activity appears to be surprisingly short. Simple fission appears to be a not uncommon mode of multiplication, and spore formation by repeated budding or by the development of a short tube, which then appears to be abscised as one or more spores, is common. Occasionally a spore develops into a promycelium of 150–250 μ with very short side branches of 4–10 μ, or less, the latter developing terminal conidia. The spores, generally ellipsoidal in form, show great variations in size and outline (Pl. XVII, fig. 3), ranging from 2.3 to 10.6 μ in length and from 1.2 to 4.2 μ in width. Such variations may perhaps be ascribed to different stages with respect to germination. Germination from the two poles is quite common (Pl. XVII, fig. 1), a fact noted by Kuehn (11). Spores are frequently septate before germination, and sometimes the wall appears after the formation of a promycelium. The bipolar granular areas noted by Brown and Ranck in spores of *C. Paspali*, and which Meyer in his study regards as oil drops in *C. purpurea*, are conspicuous (Pl. XVII, figs. 2, 7, 9).

Following the mycelial development just noted, fresh inoculations from the same source were made on the same agar media in small 50-cc. Erlenmeyer flasks and on plugs of carrot, potato, and turnip. In addition, 100-cc. Erlenmeyer flasks containing mashes of white corn meal, ground rye seed, and ground rye screenings were used in this series. The inocula were taken from the area of vermicular growth. The new growths upon agar in the flasks repeated the vermicular character until they had attained an average size of 2 to 3 cm. in diameter (Pl. XVI, figs. 1, 2). Following this stage, the surfaces of the media were gradually covered by a very thin, silvery mycelium. No aërial mycelium developed in any agar cultures for several weeks following inoculation. As growth progressed, the central areas in some agar flasks formed a pellicle, becoming raised and slightly folded and convoluted but not in a manner to form the intricate vermicular type of growth (Pl. XVI, fig. 3). The mat developed a thickness of somewhat less than a millimeter. Studies of the central and peripheral portions showed a great predominance of spores in the former and a great mycelial development in the latter.

No growth was obtained on turnip, and a very moderate vermicular growth developing sparse mycelium resulted on potato. On carrot plug growth was rapid and abundant. Within 24 hours following inoculation with a gelatinous portion of the inoculum, this had spread over three fourths of the carrot slant, and within a very few days this growth resulted in a very dense white mycelial cushion which rapidly penetrated the tissue of the plug. This mycelium conformed with descrip-

tions of Brefeld and of Meyer, showing typical hyphae and spore formation with great numbers of abscised spores arranged in parallel fashion along the threads. A similar grouping was commonly noted in growths on agar (Pl. XVII, figs. 5, 7).

Of the three growths on the mashes, that obtained on the rye screenings was an extremely sparse aërial mycelium. On the rye-seed mash a growth of the usual gelatinous-vermiculate type started, which covered the surface of the media in about a week or ten days. From this stage resulted a very dense mat of aërial mycelium, which in the course of several weeks showed a slight tendency to assume a pinkish or purplish hue. The growth upon white corn meal was so rapid and so similar in character to the surface of the corn meal after sterilization that it was difficult to note its progress until it had reached the side of the flask the second or third day after inoculation. Three weeks from the time of inoculation the entire surface and a very large portion of the medium bounded by the sides of the flask were covered with a dense mycelial growth which became strongly convoluted and took on a deep purple-black color similar to that of the sclerotia of Claviceps. As time progressed, the invasion of the fungus continued, although somewhat checked by the gradual drying of the medium. The aërial mycelium in the agar flasks, on the other hand, did not develop uniformly on all three kinds of media, or over the entire surface in any one, but appeared as isolated areas or as a ring.

On agar in Petri dishes, where the growth was confined to the surface and no pellicle of any thickness developed, there was observed the parallel massing of hyphae in strands of from two to five or six threads, which Meyer describes as a morphological variation of the organism on solid media.

We have been unable to confirm this view. No morphological relation of the fungus to solid media of any kind has been observed. On the several kinds of agar other than that in the Petri dishes, and on the other organic solid media with abundant water content, the mycelium developed the usual branching form regarded by previous workers as normal. We incline to the view that the growth variations noted may possibly be a reaction to a surface-tension factor associated with low water content of the medium.

Strands of parallel hyphae were occasionally observed in the same field with branching mycelium. There also appeared to be two types of hyphal development, the threads of one type being two to three times broader than the smaller, predominating kind. The broader filaments were in some instances split at the tips to form long sterigmata, but no spores were observed in such cases.

Our observations also conflict with Meyer's views regarding the greater age of the vacuolated mycelium. In our cultures the latter was most frequently found in the areas of earliest growth, namely in the vicinity of the inoculum. The hyphae of later development, especially the aërial

mycelia, have not, even when of considerable age, shown a marked vacuolation or massing of protoplasmic contents as a general characteristic. There appears to be a marked relation between the early gelatinous-vermiculate stage of growth and a lack of normal mycelial development. In this stage, our studies show an extremely short cycle from one spore generation to another, as previously noted. It is in this area of prolific spore production that the short, partially vacuolate hyphae have been most generally observed (Pl. XVII, fig. 1, *A*, *C*; fig. 6). The protoplasmic contents are generally segmented areas of the size and form of spores in early stages. In addition, mycelium in early stages of development has frequently been noted in which non-terminal cells were swelling and assuming the spore form (Pl. XVII, figs. 7, 8, 9). We incline to the view that the phenomena above noted may be regarded as responses by the fungus to cultural conditions and that these growth abnormalities indicate the probability of chlamydospore formation.

The final series of cultures in this preliminary study consisted of 500-cc. flasks containing mashes of white corn meal, yellow corn meal, white potato, sweet potato, carrot, rye seed, rye screenings, string bean, and rye heads after flowering (Pl. XVIII). The latter medium consisted of entire rye heads collected shortly after the flowering period, dried, and finely ground. The weight of solid material used in each case varied from 100 grams in the case of the corn meals to 300 grams for green string beans, with a corresponding variation in the amount of water added in each case, so that the respective substrates after autoclaving resulted in compact solid masses with a water content sufficiently great to overcome the desiccation factor for an extended period of growth. The flasks were inoculated in mid-September, 1920, with inocula from the vermicular area of one of the preceding agar cultures in flasks.

Response to different media was indicated by marked variation in both rate and character of growth. That on sweet potato was the most rapid in the initial stage, the entire surface of the medium being covered with very vigorous aërial mycelium in about one week. At the same time, growths were moderate on the two types of corn meal and on rye seed; string bean and white potato showed slight growth of the commonly observed gelatinous character. Carrot, contrary to the results of our previous work, showed remarkably slow growth. The flask of rye heads showed only a trace. This latter material was not in a compact form, due to its agitation as the result of autoclaving, but we succeeded at this time in shaking the material up and spreading the inoculum, so that better growth resulted thenceforward. The rate of growth after the first week did not maintain the same relative pace, and three months after inoculation the most abundant growth was again to be seen on the two corn meals. In these media the marked purple color and heaping up of the mat in vermiculate form somewhat resembling sclerotia were

very abundant (Pl. XVIII, figs. 1, 2). This stage was, of course, preceded, as in the former cultures on corn meal, by that of the dense white aërial mycelium. Next in order of luxuriance was the growth on rye-seed mash (Pl. XVIII, fig. 3). Here the convolutions of the matted surface were much larger, and there was only a hint of a tendency to purple coloration. The aërial mycelium progressed much farther down the sides of the flask than on corn meal. Sweet potato (Pl. XVIII, fig. 9) apparently suffered an inhibition of mycelium production of the aërial type, and the surface was now well convoluted, of a muddy putty color, on the surface of a considerable portion of which was a growth of very short aërial mycelium. On white potato (Pl. XVIII, fig. 6) there developed a very limited light brown, entirely vermiculate growth over the top surface, very small in scale compared to previous growths. String bean (Pl. XVIII, fig. 8) developed the vermiculate type over the entire surface, the convolutions small in scale, densely compacted, with a tendency to develop aërial mycelium, on the surface of the medium contiguous to the sides of the flask. Rye screenings (Pl. XVIII, fig. 4) produced a vermicular growth markedly raised in the center. Mycelium started on the periphery and grew down the side of the flask. The carrot culture (Pl. XVIII, fig. 7) developed no mycelium at the time indicated, was very finely vermiculate at the periphery, and had only partially covered the top surface. The central growth was pustular in character. Growth on rye heads after flowering (Pl. XVIII, fig. 5) was chiefly aërial mycelium and appeared to be working along the surface rather than permeating the mass.

From this time on growth was either extremely slow, or had apparently ceased. Six months after inoculation growth was characterized as follows:

On the corn meals the purple, heavily plicated mat developed over the entire upper surface and over portions of the sides. The earliest stage —the gelatinous—advanced along the sides well towards the bottom. Intermediate between these two was an area of aërial mycelium. The latter had also developed from the convoluted purple upper surface. There was no marked difference between growths on yellow or on white meal. On rye seed the gelatinous, coarsely plicated pellicle covered all surfaces except the base. Aërial mycelium was absent. On rye screenings a finely vermicular pellicle of light-brown color covered the upper surface with no increase of mycelial growth. No further growth was apparent on the rye-heads substrate, which was fairly dry. On white potato the vermicular growth covered the upper surface and a portion of the sides. The carrot flask showed a gelatinous, finely vermicular pellicle covering the top and sides of the substrate to its base. A very sparse, short aërial mycelium was developing in the center of the top. On the string-bean substrate a most compact, finely vermicular growth covered the top and three fourths of the sides. Aërial mycelium had started on the sides only. The flask of sweet potato had previously been broken.

The tendency of the fungus to grow upon the surface of the medium rather than to penetrate the mass to any marked degree—a fact noted by Meyer—was observed in the first series of corn-meal and rye-seed mashes studied. Most probably failure to penetrate is closely related to time and desiccation factors, since the fungus, after the initial stages, grows comparatively slowly. That this appears to be true is indicated in the 500-cc. flask of white corn meal above mentioned, from which the mass was removed *in toto* by breaking the flask, six months after inoculation (Pl. XIX, fig. 1). The corn-meal mash still contained an abundance of moisture, doubtless sufficient for indefinite growth of the fungus. Progression of the pellicle along the surfaces bounded by the flask is readily noted, and the longitudinal section through the center of the mass shows an almost solid layer of fairly compact fungous tissue 2–3 cm. in thickness (Pl. XIX, fig. 2). This is not an accretion of surface growth, since originally the surface of the medium was approximately that of the upper surface of the fungus. It is clearly a growth which has penetrated and apparently completely utilized the substrate in its metabolism.

Sections of this growth at two stages show a type of development completely at variance with Meyer's descriptions and illustrations of what we assume was a similar stage. The older or outer layers do show a denser, compacted growth, but not characteristically parallel; neither has the basidial development of Meyer's description been confirmed. Contrary to his findings that the resting hyphae are not pseudo-parenchymatic, this is precisely what the material shows. Indeed, there is a striking analogy between transverse sections of a sclerotium (Pl. XIX, fig. 6) and of the fungus in culture. There is a marked development of the surface hyphae resembling an epidermal formation (Pl. XIX, fig. 3), and an unmistakable pseudo-parenchymatic formation in the general mass (Pl. XIX, figs. 4, 5). The pseudo-parenchymatic appearance of the sclerotium is doubtless due to a mesh of hyphal elements developed under pressure. It differs in essential characters from sections through the culture chiefly in the smaller size of the cell-like areas and in the compacted cells forming the outer tissues of the sclerotium. We believe the conclusion is warranted that the fungus in culture develops a stage analogous to that found in the sclerotium and that the differences in scale of the cell-like structure are probably due to the fact that in the flask the fungus grew freely, while in the sclerotium growth is confined and subjected to pressure.

As previously stated, no attempt has been made to emphasize the mycological side of the present study, especially since our observations—with the exceptions noted—accord in the main with those of former workers in this field. On the other hand, we have found no reports in the literature dealing with the physiological properties of Claviceps cultures. Engelke's paper, so far as can be learned, does not appear to have been followed by work along the line he proposed. The chief interest in the present

work has therefore centered in the physiologically active constituents of the organism in culture, compared with those in the sclerotium of natural development.

The Chemistry and Toxicology of Ergot

The chemistry and toxicology of ergot were studied as early as the eighteenth century, but it is only within recent times that the work of Barger (2, 3, 4, 5), Dale, Ewins (10), and others has resulted in accurate knowledge.[2]

The chief active principles so far isolated from ergot are, according to Barger:

(1) Ergotoxine ($C_{35}H_{41}O_6N_5$), an amorphous alkaloid yielding crystalline salts, which in very small doses produces ataxia, dyspnoea, salivation, gastro-intestinal irritation, and gangrene. The latter is caused by constriction of the arterial circulation which this alkaloid effects.

(2) Histamine (ergamine, β-iminazolyl-ethylamine, $C_5H_9N_3$), a powerful uterine stimulant and blood-pressure depressor.

(3) Tyramine (parahydroxyphenylethylamine, $C_8H_{11}ON$), functioning as the powerful blood-pressure-raising principle in ergot extracts.

(4) Acetylcholine ($C_7H_{17}O_3N$), a depressor of blood pressure.

The standard physiological tests of ergot extract for pharmaceutical use are made for the first three of the above named constituents. Detailed methods are described by Pittenger (13). Histamine action is determined by subjecting freshly excised guinea pig's uterus, suspended in Ringer's solution, to the standard ergot dose, which is added to the solution, the resulting muscular contraction being recorded on a kymogram apparatus. For the tyramine test, cats, dogs, or rabbits are used, the drug being injected intravenously and the rise in blood pressure being recorded. Tests for ergotoxine are most generally made upon the comb of the domestic fowl. Injection is intramuscular. The action generally occurs within an hour, evidenced by a very distinct bluing of the comb and sometimes of the wattles. Attendant symptoms are restlessness, drooping of the head and tail, diarrhoea, and inability to stand normally erect.

Since the greatest development, and apparently the most advanced stages of growth, appeared in the cultures on corn meal, they were the ones selected for tests of physiological activity. For the first of these the growth on corn meal in 100-cc. flasks was used. In this instance growth did not permit a ready separation of the fungus from the substrate, and the entire mass was air-dried to constant weight, and then ground and percolated for the fluid extract. Forty-two grams of substrate yielded 10.86 grams dry weight of material for percolation. On the basis of dry weight the total fungus was estimated as not to exceed 2 percent of the

[2] An excellent summary, with bibliography, of the entire subject of ergot, embracing its history, botany, medicinal properties, aetiology, chemistry, etc., has been made by Barger (6).

total dry weight used in extraction. Test of the extract from this source in standard dose produced no distinctive bluing of the comb of a White Leghorn cock, and only a slight indication of increased blood pressure in a rabbit.

The second test was made with an extract in which the fungous material largely predominated. To attain this end, the cultures were grown on a thin layer of corn-meal mash in Petri dishes (10 g. of meal, 10 cc. of 1 percent peptone solution, and 10 cc. tap water). These cultures, inoculated from one of the 500-cc. flasks in the series previously discussed, grew rapidly, passing through all the previously described stages (Pl. XX, fig. 1) and developing at a comparatively early stage the soluble purple color (presumably sclererythrin, a physiologically inert substance). This diffused quite readily to the lower surface of the substrate (Pl. XX, fig. 2). Sterile water was added when necessary to promote growth. At the end of three months, four dishes were dried to constant weight, and their contents were removed, ground, and extracted by the standard method. The finely ground material had the general appearance and the peculiarly musty odor of ground ergot sclerotia. Forty grams of the original corn meal yielded 22.2 grams of dried powdered fungus and substrate mixture. Microscopic examination showed that this contained less than thirty per-cent (estimated) of unaltered corn meal.

In color and odor the fluid extracts from this material—as, indeed, in the one previously made, and in the one to follow—were characteristic of standard ergot extract except that in shade of color they were somewhat lighter, resembling more closely a percolate of domestic rather than of foreign ergot.

With this extract tests were made as follows:

For tyramine (parahydroxyphenylethylamine). Blood-pressure tests were made on the rabbit and cat. Negative results were obtained on a rabbit with both fungous extract and standard U. S. P. ergot extract. On cats the latter produced increase of blood pressure characteristic of standard ergot preparations. The extract of the fungous culture effected a lowering of pressure but showed no pressure increase.

For histamine. A uterine muscle-contraction test was made on guinea pig. The kymogram chart (Pl. XXI, fig. 1) shows the results obtained. *A* and *C* indicate the contraction resulting from the addition of standard U. S. P. ergot extract, 1–10,000. This represents 1 gram of powdered ergot per 10,000 cc. solution. *B*, *D*, and *E* are the contractions obtained with extracts from the culture in strengths of 1–3300, 1–5000, and 1–7000, respectively. The figures beside each wave are the distances in millimeters from crest to base line.

It is clear that there was present in the extract a principle causing uterine contraction and ranging in strength about from 1/3 to 7/10 that of standard ergot preparations.

For ergotoxine. The tests for this alkaloid on White Leghorn cocks were in all cases negative, so far as indices of comb-bluing are concerned. Two tests were made on a bird previously standardized for his reaction to U. S. P. ergot, the injections being made at intervals of four or five days to permit complete recovery from the previous doses. The dose was in each instance 2½ times the strength necessary to produce a very distinct comb-bluing of the same bird with standard ergot extract. In neither case did the injection of culture extract produce anything more than a very slight bluish tinge of portions of two tips of the comb. The slight color changes may well be ascribed to the general physiological reaction of the fowl to the injection, as the comb is a fairly sensitive index of condition, but no deductions other than negative are warranted with respect to ergotoxine action.

On the other hand, there was clear evidence of toxaemia. One half hour after injection the bird was distinctly sick, exhibiting general excitation, drooping of head and tail, and shaking of comb. Diarrhoea was noted in both trials about one half hour after injection, in one instance being more pronounced than in the other. One hour after injection the bird was very weak on its legs (Pl. XXI, figs. 3, 4), inclined to squat or to lie flat, with sunken head (Pl. XIX, fig. 5). A complete return to normal condition was not effected for several days.

A final trial of extract of pure fungus was made with material taken from abundant growth in the 500-cc. flask of corn meal illustrated in Plate XIX, figures 1 and 2. With the exception of a small portion reserved for microscopic study, the entire upper layer of fungus here shown was removed and extracted after drying. Fifty-six and eight tenths grams of fungus produced 5.8 grams of air-dried material for extraction. The latter had the same gross characters as previous material. A dose seven times the normal produced no effects of any kind in a healthy cock. The extract, when tested chemically, showed no trace of alkaloids.

Controls with extracts of autoclaved and dried corn meal were carried on with the foregoing tests, and in all cases gave negative results.

Discussion

The present study confirms in great part the descriptions by former students of *C. purpurea* in culture, but opposes them in several particulars —notably in regard to the question of morphological variations and the development in cultures of advanced age of a stage distinctly analogous to the pseudo-parenchyma and epidermal layers of the natural sclerotium. The development of the conidial stage directly from the sclerotium without germination and without formation of ascospores has been demonstrated.

The data presented give indications of the presence in Claviceps cultures as here grown of but one of the commonly recognized active principles of ergot—histamine. There is at present no ground, however, for a belief

in the presence of ergotoxine in the cultures, in the light of chemical examination. The qualitative test was made upon a very small amount of material, so that analyses upon a larger scale will be necessary before this fact will be established with certainty. It is highly possible, however, that the presence of the alkaloid is directly associated with changes involving sclerotial formation, and the present work conforms with that of its predecessors in failure to obtain this resting stage under cultural conditions.

In the light of the present study it appears extremely doubtful that the artificial culture of Claviceps possesses practical application. There are several factors indicated by this preliminary work which need to be determined before a definite conclusion in this respect may be drawn.

(1) The organism must be grown in quantities sufficient to make practicable a careful quantitative and qualitative study of the physiologically active principles present.

(2) The marked variation in growth response to different media indicates that its behavior and constitution under cultural conditions of known $_{P}H$ values would afford a valuable field of investigation.

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REFERENCES

1. **Atanasoff, D.** Ergot of grains and grasses. Bur. Pl. Ind. U. S. Dept. Agr. Pp. 127. 1920.
2. **Barger, G., and Carr, F. H.** The alkaloids of ergot. Jour. Chem. Soc. **91**: 337–353. 1907.
3. ——, **and Dale, H. H.** Ergotoxine and some other constituents of ergot. Biochem. Jour. **2**: 240–299. figs. 1–8. 1907.
4. ——. Isolation and synthesis of p-hydroxyphenylethylamine, an active principle of ergot soluble in water. Jour. Chem. Soc. **95**: 1123–1128. 1909.
5. ——, **and Ewins, A. J.** The alkaloids of ergot. Part 2. Jour. Chem. Soc. **97**: 284–292. figs. 1–5. 1910.
6. ——. Ergot and ergotism in the past. Pharmaceut. Jour. **105**: 470–473. 1920. (Also in Nat. Druggist **51**: 82–84, 141–142. 1921.)
7. **Brefeld, O.** Untersuchungen aus dem Gesammtgebiete der Mykologie (Ascomyceten II) **10**: 192, 193. Pl. 5, figs. 63–67. 1891.
8. **Brown, H. B., and Ranck, E. M.** Forage poisoning due to *Claviceps Paspali* on Paspalum. Miss. Agr. Exp. Sta. Tech. Bull. 6. Pp. 35, figs. 1–18. 1915.

9. **Engelke, C.** Neue Beobachtungen über die Vegetationsformen des Mutterkornpilzes *Claviceps purpurea* Tulasne. Hedwigia **41**: 221, 222. 1902.
10. **Ewins, A. J.** Acetylcholine, a new active principle of ergot. Biochem. Journ. **8**: 44–49. 1914.
11. **Kuehn, J.** Über die Entstehung des Mutterkorns. Ber. Sitz. Naturforsch. Ges. Halle 12–14. 1863.
12. **Meyer, B.** Untersuchungen über die Entwickelung einiger parasitischer Pilze bei saprophytischer Ernährung. Landw. Jahrb. **17**: 915–945. Pls. 16–19. 1888.
13. **Pittenger, P. S.** Biochemic drug assay methods. Pp. 158, 88 fig. 1914.
14. **Stäger, R.** Infectionsversuche mit überwinterten Claviceps-conidien. Mycol. Centralbl. **1**: 198–201. 1912.

EXPLANATION OF PLATES

PLATE XVI

Growths of *Claviceps purpurea*

FIG. 1. On rye-screening agar, early stage.
FIG. 2. On rye-screening agar, late stage.
FIG. 3. On rye-seed agar, late stage. Photographed when agar had begun to shrink from sides of flasks. Appearance of growths unaffected.

PLATE XVII

FIG. 1. Germinating spores. *A–C*, × 900; *D*, × 385.
FIG. 2. Spores, rye-screening agar, vermiculate area. × 900.
FIG. 3. Spores, rye-seed agar, vermiculate area, later stage. × 900.
FIG. 4. Mycelium, Petri dish culture, rye-seed agar. Full size.
FIG. 5. Mycelium and spores, rye-screening agar. × 900.
FIG. 6. Mycelium on carrot. × 385.
FIG. 7. Mycelium on rye-screening agar. × 900.
FIG. 8. Mycelium on carrot. × 385.
FIG. 9. Mycelium on rye-screening agar. × 900.

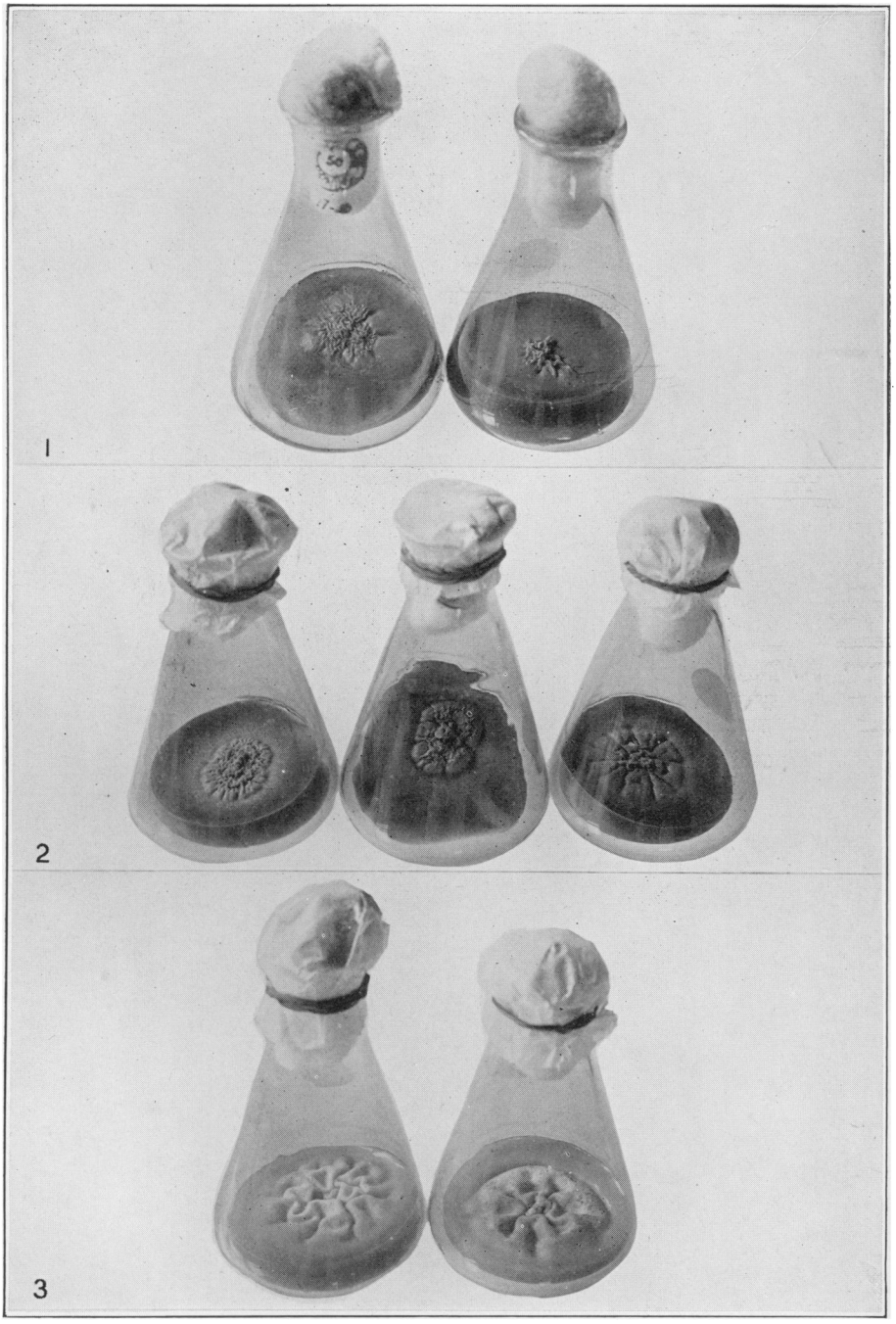
PLATE XVIII

Growths on mashes after three months

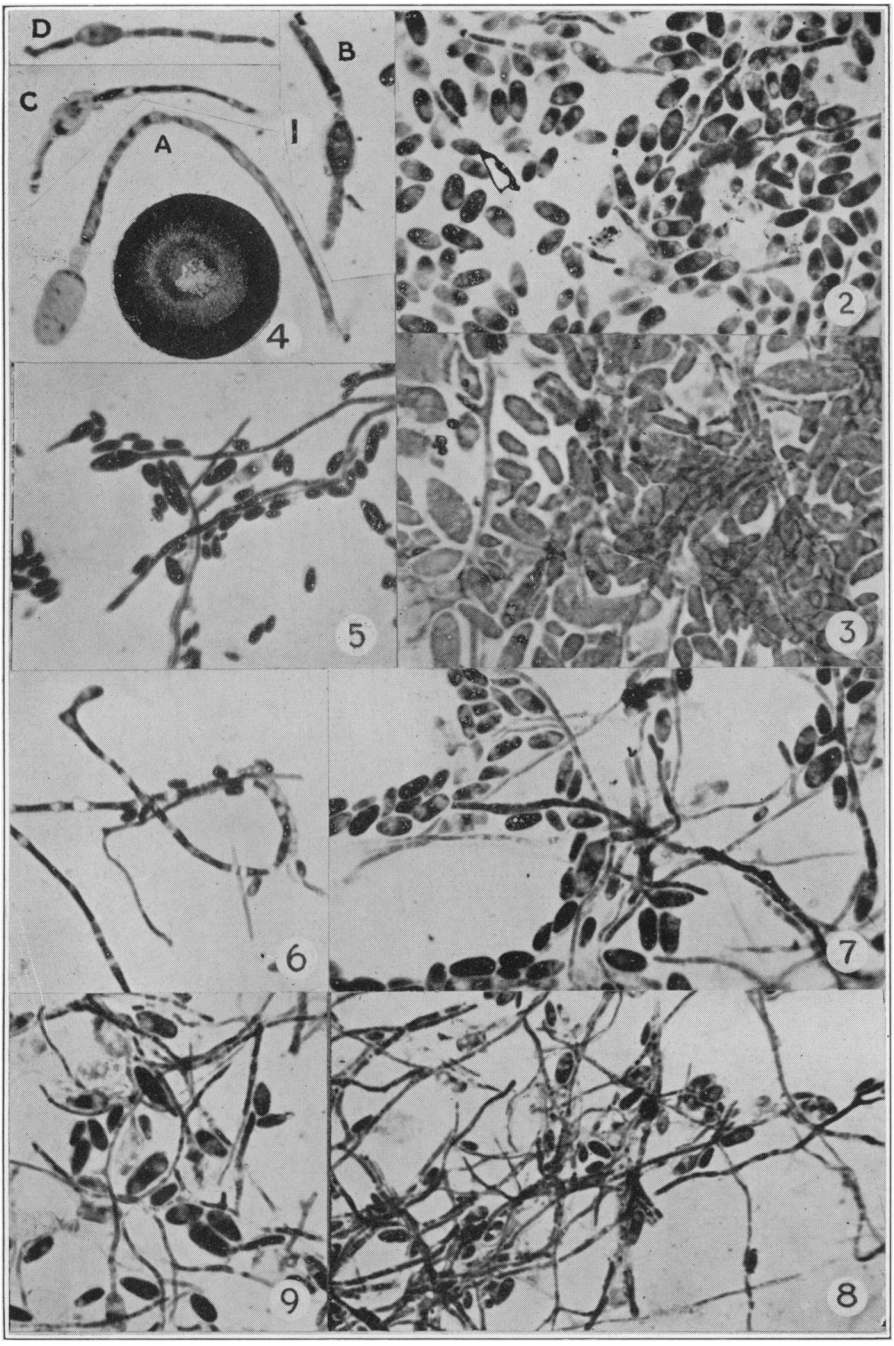
FIG. 1. On white corn meal.
FIG. 2. On yellow corn meal.
FIG. 3. On rye seed.
FIG. 4. On rye screenings.
FIG. 5. On rye heads after flowering.
FIG. 6. On potato.
FIG. 7. On carrot.
FIG. 8. On string bean.
FIG. 9. On sweet potato.

PLATE XIX

FIG. 1. Six months' growth on white corn meal.
FIG. 2. Longitudinal section through same.
FIG. 3. Section through surface portion of growth in the same flask, showing tendency to epidermal formation. × 200 (approximate).
FIGS. 4, 5. Sections through center of growth, showing pseudo-parenchyma. × 200 (approximate).
FIG. 6. Section of ergot sclerotium. × 200 (approximate).



BONNS: CLAVICEPS PURPUREA



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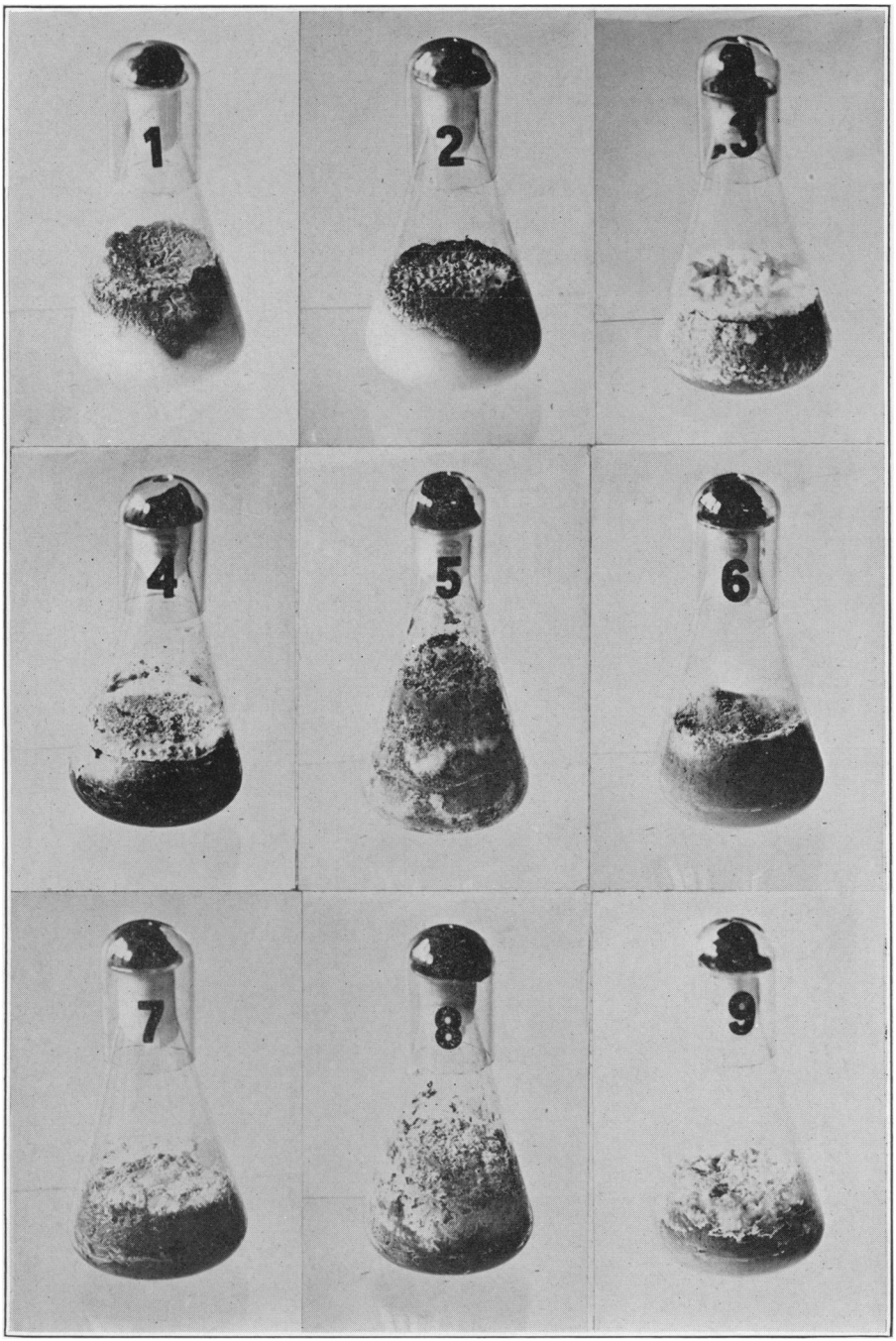

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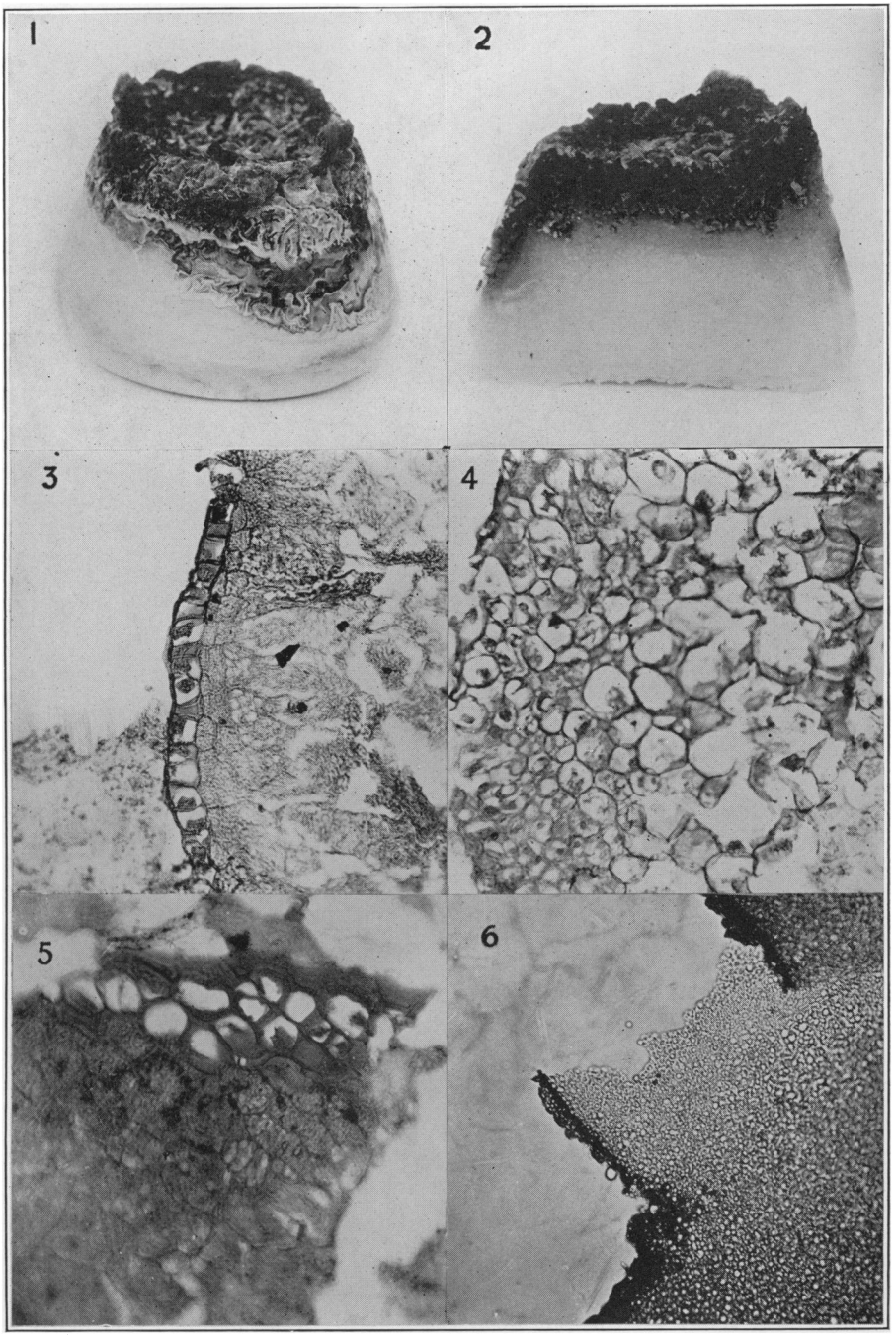

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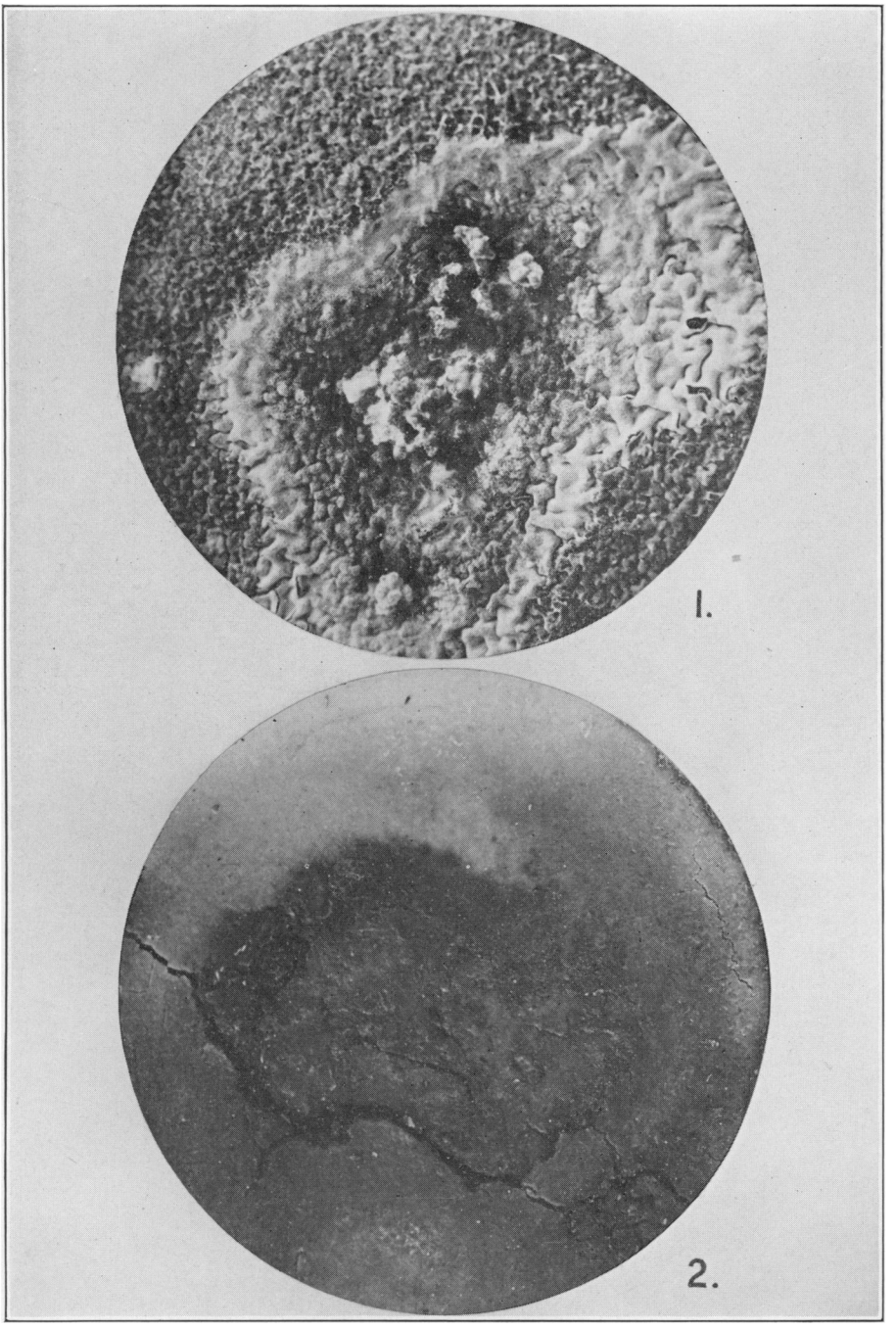

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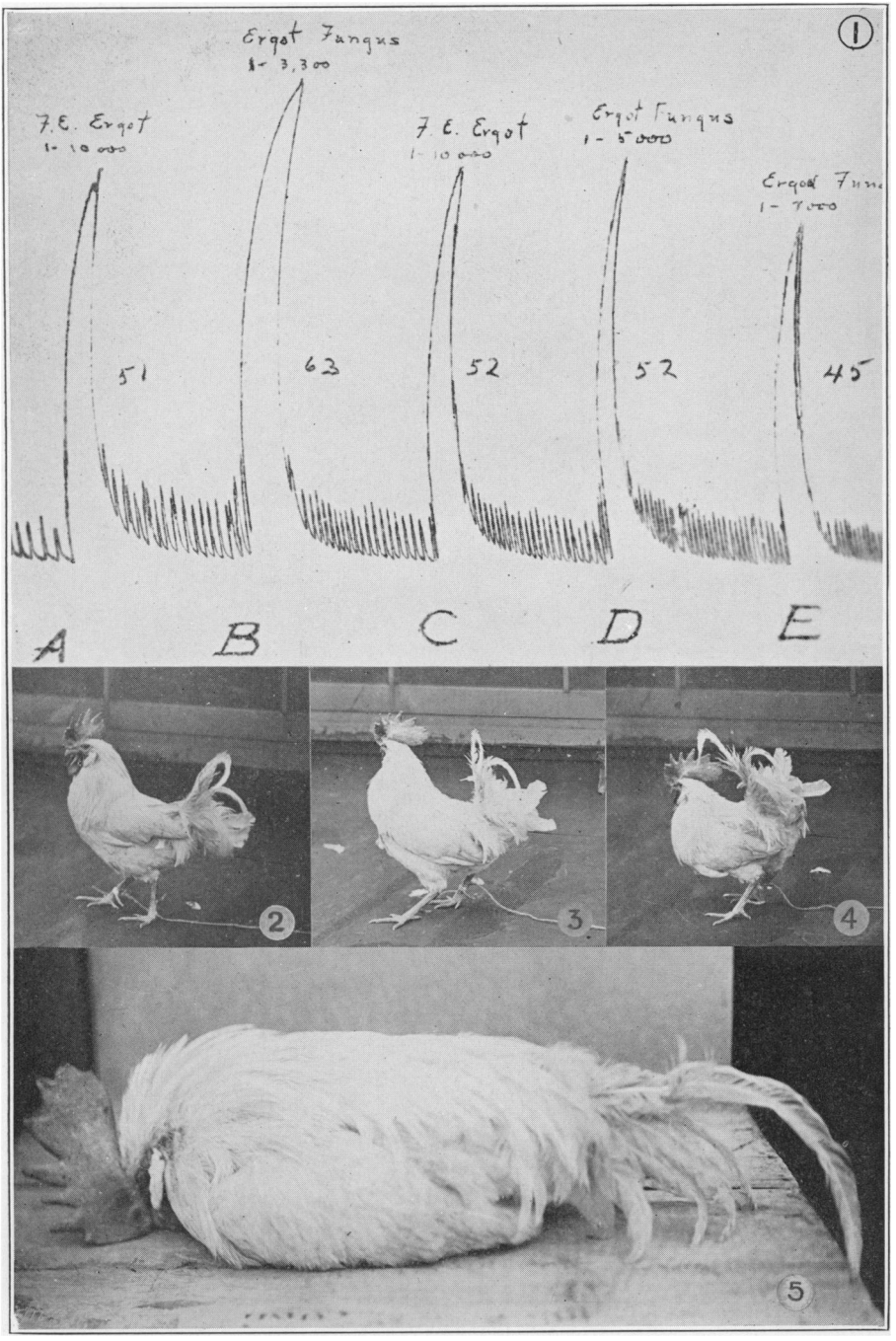

BONNS: CLAVICEPS PURPUREA

PLATE XX

Growth on corn meal in Petri dish

FIG. 1. Top.

FIG. 2. Bottom, showing diffusion of pigment through substrate.

PLATE XXI

FIG. 1. Kymogram record, showing contraction effect of standard fluid extract of ergot (*A* and *C*), and of extract from Claviceps cultures (*B*, *D*, and *E*), at different dilutions, on uterine muscle of guinea pig. Figures give height in millimeters of wave from base line to crest. Extract concentrations at crests.

FIG. 2. Normal posture of White Leghorn cock.

FIGS. 3, 4, 5. Effect of Claviceps culture extract on posture of White Leghorn cock.